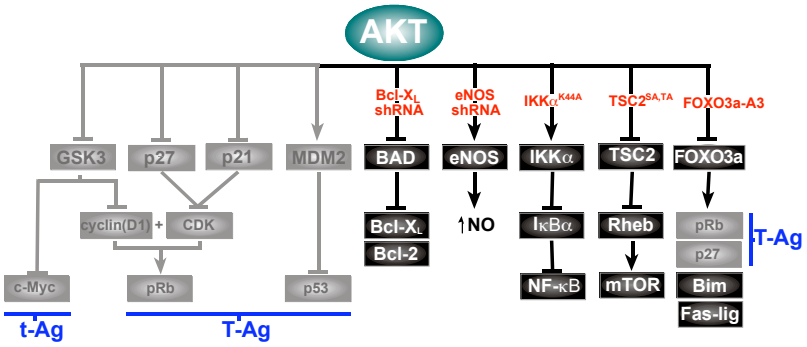
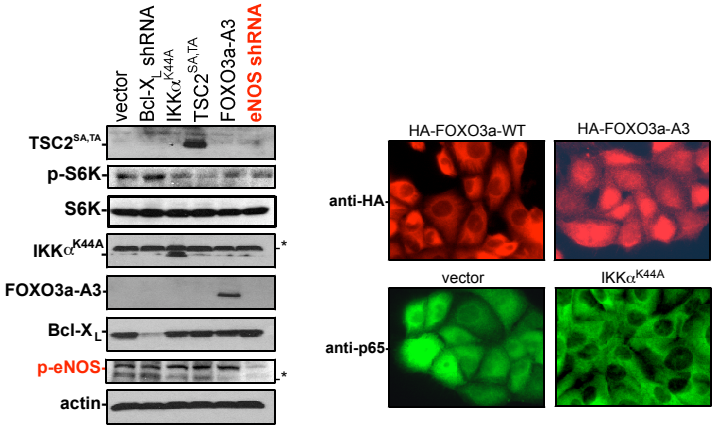
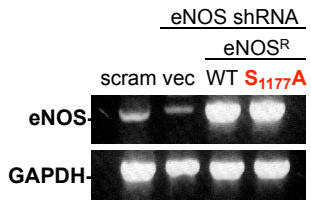


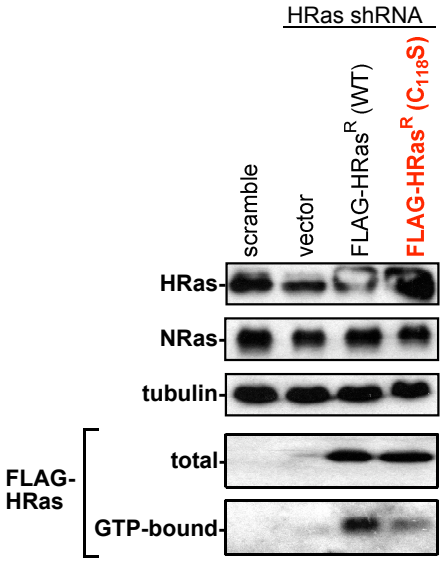
Supplemental Figure 1 | Pathways explored downstream of AKT. Light boxes, pathways altered by T-Ag or t-Ag expressed in TtH cells used in the cell-mixing assay of tumour maintenance. Black boxes, pathways altered using the indicated (red lettering) molecules (see reference 4).



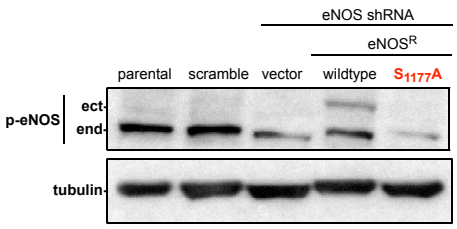
Supplemental Figure 2 | Confirmation of disruption of pathways downstream of AKT. (***Left***) Protein levels assessed by immunoblot of the indicated proteins in PI3K-TtH^{LacZ} cells expressing the indicated proteins or shRNA. *non-specific bands. (***Right***) Subcellular localization and protein analysis by immunofluorescence. The indicated transgenes were expressed in PI3K-TtH^{LacZ} cells. Mutant HA-FOXO3a-A3 demonstrates appropriate localization to the nucleus, and expression of mutant IKKα^{K44A} results in exclusion of p65 from the nucleus.



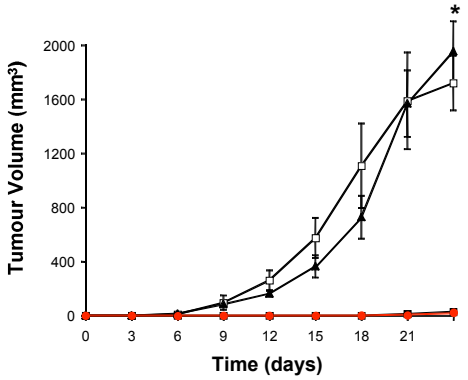
Supplemental Figure 3 | Confirmation of eNOS knockdown and complementation by eNOS^R proteins. mRNA levels assessed by RT-PCR of eNOS in PI3K-TtH^{LacZ} expressing scramble control or eNOS shRNA and complemented with either vector or shRNA-resistant eNOS (eNOS^R) in the wildtype (WT) or $S_{1177}A$ mutant configuration. GAPDH: RT-PCR control.



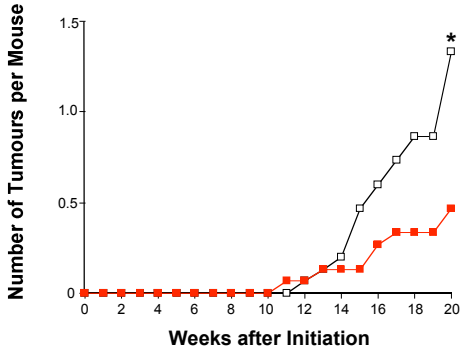
Supplemental Figure 4 | Confirmation of HRas knockdown and complementation by HRas^R proteins. Protein levels assessed by immunoblot of endogenous HRas and NRas, total or GTP-bound wildtype (WT) or $C_{118}S$ shRNA-resistant FLAG-HRas^R in PI3K-TtH^{LacZ} cells expressing a scramble control sequence of HRas shRNA and the indicated trangenes. Tubulin serves as a loading control.



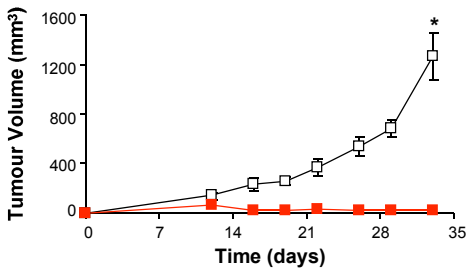
Supplemental Figure 5 | Confirmation of eNOS knockdown and complementation by eNOS^{R} proteins. Protein levels assessed by immunoblot of ectopic (ect) and endogenous (end) S1177-phosphorylated eNOS (p-eNOS) in Ras^{G12V}-TtH cells expressing a scramble control sequence or eNOS shRNA with vector, wildtype, or $S_{1177}A$ RNAi-resistant eNOS (eNOS^{R}). Tubulin: loading control.



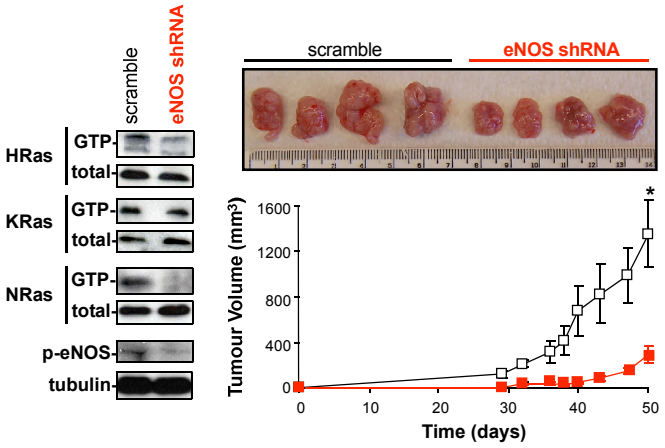
Supplemental Figure 6 | Tumour growth depends upon S_{1177} of eNOS. Tumour volume versus time of Ras^{G12V}-TtH cells expressing scramble (□) or eNOS shRNA with vector (■), wildtype (▲) or $S_{1177}A$ (●) RNAi-resistant eNOS. n=4, error bars = s.e.m., *p<0.001: (□) versus (●).



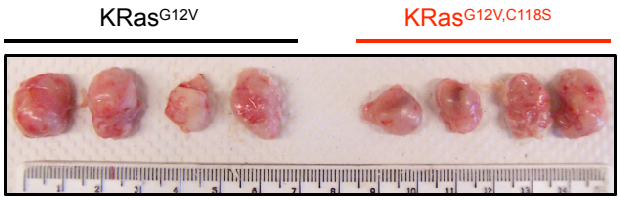
Supplemental Figure 7 | *eNOS*$^{-/-}$ mice have fewer tumours induced by DMBA/TPA. Mean number of tumors per *eNOS*$^{+/+}$ (□) versus e*NOS*$^{-/-}$ (■) mouse versus time after initial application of DMBA. n=15, *p<0.01.



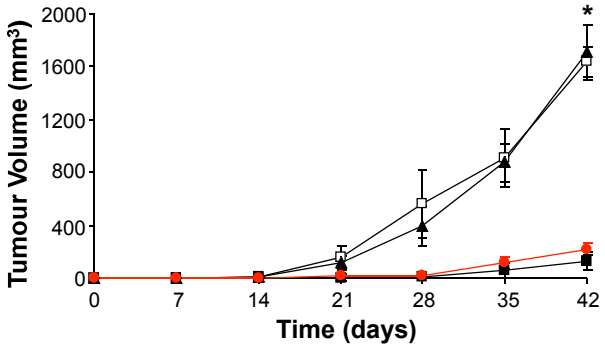
Supplemental Figure 8 | Knockdown of eNOS inhibits tumor growth of CFPac-1 cells. Tumour volume versus time of CFPac-1 cells expressing scramble (□) or eNOS shRNA (■). n=4, error bars=s.e.m., *p<0.01.



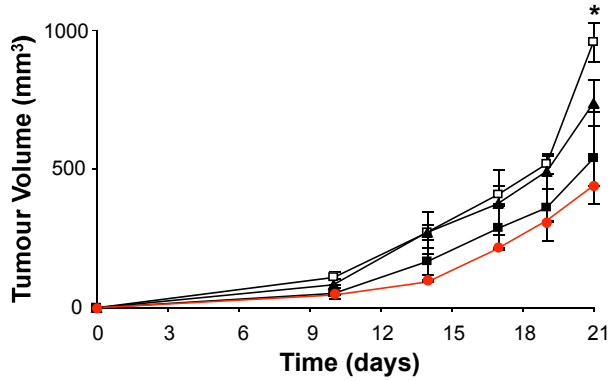
Supplemental Figure 9 | Knockdown of eNOS inhibits tumour growth of MIAPaCa-2 cells. ***Left,*** Protein levels assessed by immunoblot of GTP-bound endogenous HRas, KRas, and NRas, and phosphorylated eNOS (p-eNOS) in , and as loading controls,total HRas, NRas, and HRas, and tubulin (***left***) and excised tumours or tumour growth versus time (***right***) of MIAPaCa-2 cells expressing a scramble control sequence (☐) or eNOS shRNA (■). n=4, error bars=s.e.m *p<0.05.



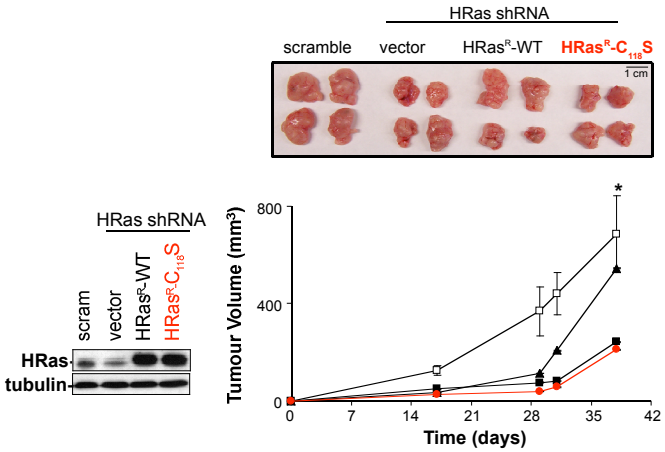
Supplemental Figure 10 | Oncogenic KRas with a $C_{118}S$ mutation remains tumorigenic. Excised tumours of TtH cells expressing $KRas^{G12V}$ or $KRas^{G12V,C118S}$.



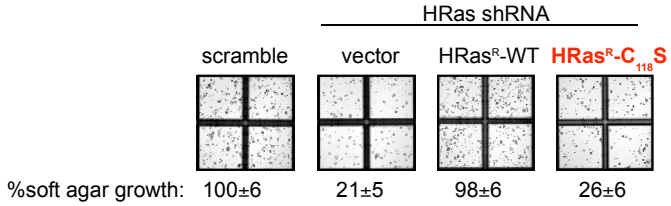
Supplemental Figure 11 | Tumour growth of CFPac-1 cells depends upon C_{118} of HRas. Tumour volume versus time of CFPac-1 cells expressing scramble (☐) or HRas shRNA with vector (■), wildtype (▲) or $C_{118}S$ (●) RNAi-resistant HRas. n=4, error bars=s.e.m., *p<0.001: (☐) versus (●).



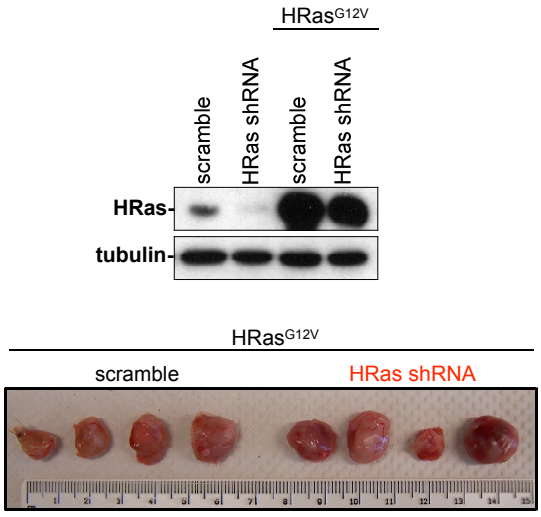
Supplemental Figure 12 | Tumour growth of CFPac-1 cells depends partly upon C_{118} of NRas. Tumour volume versus time of CFPac-1 cells expressing scramble (☐) or NRas shRNA with vector (■), wildtype (▲) or $C_{118}S$ (●) RNAi-resistant NRas. n=4, error bars=s.e.m., *p<0.001: (☐) versus (●).



Supplemental Figure 13 | Tumour growth of MIAPaCa-2 cells depends upon C_{118} of HRas. Protein levels assessed by immunoblot of HRas, or tubulin as a loading control (***left***), and excised tumours and tumour growth versus time (***right***) of MIAPaCa-2 cells expressing a scramble control (scram, □) or HRas shRNA in conjunction with vector control (vector, ■) or FLAG-HRas engineered to be resistant to RNAi ($HRas^R$) in the wildtype (WT, ▲) or $C_{118}S$ mutant ($C_{118}S$, ●) configuration. n=5, error bars=s.e.m. *p<0.05: (□) versus (●).



Supplemental Figure 14 | HRas is required for anchorage independent growth of CFPac-1 cells. CFPac-1 cells expressing a scramble control or HRas shRNA in conjunction with vector control or HRas engineered to be resistant to RNAi ($HRas^R$) in the wildtype (WT) or $C_{118}S$ mutant configuration were assayed for growth in soft agar (% of scramble control colonies±s.e.m.,from duplicate experiments in triplicate).



Supplemental Figure 15 | Loss of wildtype HRas does not inhibit oncogenic $HRas^{G12V}$-mediated tumourigenesis. Protein levels assessed by immunoblot of HRas, or tubulin as a loading control (***top***), and excised tumours (***bottom***) of TtH cells expressing either scramble or HRas shRNA in addition to RNAi-resistant oncogenic $HRas^{G12V}$.